CXLVII. INVESTIGATION OF BARLEY, MALT AND BEER FOR VITAMINS B AND C.

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(*Received July 21st, 1924.*)

SOME years ago [1918] we had the opportunity of investigating beer, malt and barley for their antineuritic and antiscorbutic potencies. The development of the study of vitamins during the intervening period has made it necessary to repeat and amplify our inquiry on this subject and this supplementary investigation forms the subject of the present communication.

BARLEY AND MALT.

(*a*) VITAMIN B.

In our original investigation we found that when malt was given to pigeons as the sole source of the antineuritic factor the birds grew but eventually developed polyneuritis. This experiment has now been repeated on pigeons as well as on rats.

Experiments on Pigeons.

In the bird experiment not only was the malt tested out but the original barley and the grain in the intermediate stages of the malting process were also investigated.

For this purpose Mr E. H. Ludlow of the Mortlake Brewery (Messrs Watney, Coombe, Reid & Co.) was kind enough to arrange throughout the period of the experiment (12. xii. 21–14. ii. 22) a continued series of maltings of a Chilian barley, so that fresh samples of the intermediate stages of the malting process could be obtained at intervals of 2 days. The intermediate stages selected were (*a*) after steeping, (*b*) after 5 days on the floor, (*c*) just before kilning, and in addition the original barley, the finished malt and the culms were examined.

Six pigeons were fed on the grain of each of the above stages, which was given to them *ad lib.* The birds on the steeped grain, 5 days germinated grain, and the germinated grain before kilning throve, gained in weight, and failed to develop polyneuritis after 2 months. The pigeons on the barley and on the malt did not gain in weight but were also protected from the disease. The group of birds on the culms alone died of polyneuritis within a month, manifesting a loss of weight.

As these results differed from that previously obtained with regard to the antineuritic properties of malt, a number of other samples of malt, dried off at various temperatures, were also tested on pigeons.

We are also indebted to Messrs Watney, Coombe, Reid & Co. for these samples.

They were marked:

Malt	Kilning temperature
(1) English Pale	195° F.
(2) „ K.K.K.	216°–220° F.
(3) Californian	195° F.
(4) English Amber	216°–220° F.
(5) „ X	200° F.

Six pigeons were fed *ad lib.* on each of these samples; nearly all gained in weight, all throve well, and did not develop symptoms of polyneuritis in 3 months, after which the experiment was terminated.

Experiments on Rats.

Barley and malt were also tested on rats. Sets of six animals were placed on diets free from the antineuritic factor (vitamin B) containing 10, 20, 30 and 50 % barley or malt respectively (3. iv. 23–1. vi. 23). The materials used were Californian barley and the malt prepared from it. These were also kindly supplied by Messrs Watney, Coombe, Reid & Co. The vitamin-free diet was compounded as follows:

Basal mixture	55 %
Purified butter fat	10
Decitrated lemon juice	27
Hardened cotton seed oil	8

The basal mixture consisted of 20 % purified casein (extracted with hot 90 % alcohol), 75 % of starch and 5 % salt mixture. Six control rats on this diet deteriorated and succumbed to the deficiency within a month.

The rats in every group receiving the malt gained in weight and survived for 2 months, after which time the experiment was terminated. The animals in the 10 % group gained on the average 25 g. during the period, those in the 20 % 30 g., in the 30 % 50 g. and in the 50 % 80 g.

Four groups of rats (six animals in each group) receiving the basal diet together with admixtures of 10, 20, 30, 50 % of the original barley from which the malt was made behaved in precisely the same manner as those animals which received the malt.

The pigeon and rat experiments both show that malt as a rule is decidedly active. It appears, however, that the antineuritic activity of malt may depend on the sample since in our original investigation no such activity could be established.

(*b*) Antiscorbutic factor (vitamin C).

In our original communication we found that malt does not possess any antiscorbutic activity, and since barley on germination acquires such activity [Fürst, 1912] we thought it advisable to find at what stage of the malting process it first appears and when it disappears. We, therefore, examined the grain for the antiscorbutic factor in the same stages of the process as we used for the antineuritic factor. Six guinea-pigs were used in each group, and were placed on an *ad lib.* ration of the grain and 40 cc. of autoclaved milk per day. As was to be expected, the barley and malt were found totally inactive. The culms had a toxic action on the animals and, therefore, the results from this group were inconclusive. The animals on the grain after steeping, after 5 days on the floor, and just before kilning, throve well on the diet and were totally protected from scurvy for 2 months, after which time the experiment was terminated. The guinea-pigs on the active grains consumed more food than those on the barley and the malt (about 15 g. per day) but when one takes into consideration the difference in the moisture content this disparity in consumption of the food is not very great and could in no way influence the results obtained. That the barley should become active so early in the process as after the steeping is very striking.

BEER.

(*a*) Antiscorbutic factor (vitamin C).

We previously found that fined beer did not contain the antiscorbutic and antineuritic factors. In testing it for the antiscorbutic factor the beer was fed to guinea-pigs both liquid and as dry residue. It was found that it did not prevent or even delay the onset of scurvy in these animals. Fresh beer was also tested on a monkey subsisting on a scorbutic diet and in this case as well the animal succumbed to scurvy after 4 months. A great number of monkeys have since been employed in this laboratory for testing the antiscorbutic activity of various substances and in no case was it found that the period required to induce scurvy in these animals was longer than about 10–12 weeks. It was, therefore, thought desirable to repeat the beer experiment on a monkey in order to ascertain whether the somewhat unusual delay observed in our first experiment in the onset of the disease was due to any antiscorbutic activity of the beer. Beer (India Pale Ale) was therefore again administered to two monkeys (*Macacus rhesus*) subsisting on a scorbutic diet which consisted of rice, casein, marmite and additional salts with the occasional addition of cod liver oil. 200 cc. of the beer was offered daily. The quantity consumed by the animals varied but the average consumption was about 150 cc. per day. One animal showed well-defined symptoms of scurvy after 37 days, the other after 57 days. This definitely confirms the observation that no appreciable antiscorbutic potency is present in the beer we have examined.

(*b*) Vitamin B.

Our previous experiments with pigeons had shown that the residue obtained by evaporating beer under diminished pressure had no antineuritic potency. A further test has now been made on the effect of a similar residue on the growth of rats. About 20 gallons of India Pale Ale, supplied by the kindness of Messrs Watney, Coombe, Reid & Co., was evaporated at 65–70° in a small vacuum pan. Particular care was taken that the beer should be bright and free from yeast, as the latter of course is known to be rich in vitamin B.

Five groups of rats of six animals in each set were employed. The constitution of the vitamin-free diet was the same as that which we employed in the tests carried out on the malt. 5, 10, 15, 20 and 25 % of dried beer was incorporated in the diet of the respective groups. The first three groups of animals all succumbed within 4 weeks. The two last groups lived a little longer, namely, about 5 weeks. This slight delay could not be attributed to the presence of any traces of the vitamin since some of the control animals subsisting on the basal diet alone also lived as long.

This confirms our previous result that the beer examined by us did not contain vitamin B.

Summary.

1. The vitamin B of barley is not affected by the malting process and is frequently present in the finished malt, but is absent from the culms.

2. The antiscorbutic vitamin appears in the steeped grain and is present in the green malt but absent from kilned malt.

3. The beer examined by us was free from both vitamins B and C.

Our thanks are due to the Directors of Messrs Watney, Coombe, Reid & Co. for their kindness in supplying the materials employed and to Messrs J. L. Baker and E. H. Ludlow for their personal assistance. Our thanks are also due to the Medical Research Council for a whole time grant to one of us.

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